Development and Resuscitation of a Sedated, Mature Male Miniature Swine Severe Hemorrhage Model

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Background: A sedated, mature male miniature swine hemorrhage model has been specifically developed to evaluate resuscitation products for the Defense Advanced Research Projects Agency Surviving Blood Loss program.

Methods: Animals were placed in a sling, sedated with midazolam, and hemorrhaged 60% of estimated blood volume (~39 mL/kg) exponentially for 1 hour with no resuscitation (control; n = 16). An additional 26 swine were treated similarly, then resuscitated with 1 mL/kg/min of Hextend to a systolic blood pressure of either 65 mm Hg ± 2 mm Hg (n = 7) or 80 mm Hg ± 5 mm Hg (n = 7) and with 17β-estradiol (E2) at 1 mg/kg (n = 6) or 10 mg/kg (n = 6). Animals were observed for 3 hours with periodic blood sampling. Survival times for the two E2 groups were not significantly different ($p = 0.59$); therefore, the groups were combined for comparison with control.

Results: Hemorrhage resulted in a characteristic hypotension and metabolic acidosis. Survival time for the control swine was 64 minutes ± 11.5 minutes with a 6% survival at 180 minutes. The 180 minutes Hextend survival was 86% for 65 mm Hg and 100% for 80 mm Hg. E2 survival was 125 minutes ± 15.3 minutes, significantly different from control ($p = 0.01$), but E2 survival of 25% at 180 minutes was not different from control.

Conclusion: A sedated, sexually mature male miniature swine severe hemorrhage model has been successfully developed, resuscitated with Hextend and used to evaluate E2 as a small volume resuscitation product.

Key Words: 60% Hemorrhage, Unanesthetized, Hextend, 6% Hydroxyethyl starch, Colloid, 17β-estradiol.

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Serious injuries often result in critical loss of blood. Immediate resuscitation with blood products is not always an option, and other resuscitation fluids such as lactated Ringer's or normal saline may require large volumes, which may not be readily available. Small volume resuscitation products are being experimentally evaluated as prehospital treatment to increase survival time for evacuation, triage, and subsequent treatment.[1]

A new hemorrhage model has been developed at the request of the Defense Advanced Research Projects Agency for testing new and novel resuscitation products for the Surviving Blood Loss (SBL) program. The model is a sedated, mature male miniature swine, 35 kg to 45 kg, socialized to human and laboratory activity and trained to lie quietly in a sling with feet on the floor. The swine was hemorrhaged 60% of estimated blood volume (EBV) exponentially for 1 hour with the anticipation of death within 1 hour after hemorrhage, without resuscitation. The SBL program intends to use this model to evaluate new resuscitation products, previously evaluated in rodents, to extend survival time after critical blood loss on the battlefield or in the civilian environment, for evacuation, triage, and supportive treatment. The sedated, mature male miniature swine was chosen for two reasons: (1) the majority of casualties are conscious mature males; and (2) the use of sexually mature domestic swine is prohibitive because of their weight at sexual maturity (100–110 kg). At sexual maturity, the miniature swine weighs 16 kg to 22 kg, and at the experimental weight of 35 kg to 45 kg, its age is 10 months to 12 months.

Both anesthetized and unanesthetized immature domestic swine have been successfully used for hemorrhage studies since the 1980s,[2–4] demonstrating that a hemorrhage of 50% to 60% of EBV was 100% lethal in swine without resuscitation but had a survival rate of 95% with aggressive resuscitation. More recently, anesthetized Yucatan male and female miniature swine (25–40 kg) were hemorrhaged 40% to 55% of EBV for 15 minutes and used to evaluate resuscitation with a hemoglobin based oxygen carrier.[5–7] Many of the techniques and solutions developed in the earlier swine studies are now in use clinically.

The efficacy of E2 has not been evaluated in a sexually mature large animal model. E2 interacts with intracellular and membrane estrogen receptors (ERs) in an integrated manner to produce both genomic and nongenomic effects. In general, genomic activity results in transcriptional upregulation or

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downregulation of numerous cellular functions, which are usually slow onset, long-term sustained effects. On the other hand, nongenomic effects are usually at the cellular membrane or vascular endothelium and are short term, relatively rapid effects, such as vascular relaxation or vasodilation resulting from acute treatment with E2.[8–13]

Female rodents (rats/mice) in estrus or castrated male rodents have an improved immune response and survival after trauma-hemorrhage (T-H) or sepsis compared with mature male or ovariectomized/diestrus female rodents.[14–16] The immune response and survival become equivalent to the estrus female or castrated male if the mature male or ovariectomized/diestrus female rodents are administered E2 before, during, or after T-H.

It is anticipated that during military operations recovery and transport of injured patients to trauma treatment areas and to the operating room table can take at least 180 minutes. Therefore, the fast, nongenomic influence of E2 on survival of the swine hemorrhage model up through 180 minutes was a major objective of this study.

The purpose of this study was to establish a spontaneously breathing, sedated, mature male miniature swine hemorrhage model defined by the Defense Advanced Research Projects Agency requirements and demonstrate that this model could be sustained for 180 minutes by using a standard of care fluid. The model could then be used to evaluate the SBL program resuscitation products. 17β-estradiol was selected for investigation because it was the most mature of the SBL program resuscitation compounds, and it is approved by the Food and Drug Administration with an extensive safety profile.

MATERIALS AND METHODS

These investigations were approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research, Fort Sam Houston, TX. All animals received care in strict compliance with the 1996 *Guide for the Care and Use of Laboratory Animals* by the National Research Council and were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility.

Hemorrhage Model Development

Healthy, mature, nonsplenectomized, male Sinclair miniature swine with a mean weight of 40.4 kg $\pm$ 1.4 kg (standard error of the mean [SEM]) were obtained from Sinclair Research Center, Inc., Columbia, MO. Health of the animal was determined with a physical examination by a veterinarian and a blood sample for basic laboratory parameters (complete blood count/blood chemistry). The animals were socialized to human activity, transport cages, laboratory procedures, and trained to lie quietly in a sling. Food was withheld 12 hours before study with water provided ad libitum. On the morning of study, each animal received 0.05 mg/kg Buprenorphine, intramuscularly (i.m.), followed by 4 mg/kg to 5 mg/kg Telazol, i.m., 30 minutes to 60 minutes later. When the swine was adequately sedated, isoflurane at 1% to 5% in 100% O_2 was administered using a face mask cone, until a surgical plane of anesthesia was obtained. The animal was placed prone and intubated with a 7-mm to 8-mm endotracheal tube, and anesthesia was continued using a Datex-Ohmeda Aestiva/5 anesthesia machine (Madison, WI), maintaining an approximate end-tidal CO_2 of 40 mm Hg. An ear vein was cannulated with an 18- to 22-gauge intracath for subsequent midazolam administration. The animal was rotated onto its back and shaved in preparation for surgical placement of catheters. A cut down was made over the right external carotid and the right lower thigh femoral area. A small branch of the external carotid artery was cannulated with a Data Sciences International (DSI, St. Paul, MN) telemetry pressure transducer catheter for the measurement of arterial blood pressure. If an ear vein was not available for cannulation, then the external jugular vein was cannulated with a silastic catheter (0.062 inch inside diameter $\times$ 0.125 inch outside diameter) for midazolam administration. Similar silastic catheters were placed in the distal femoral artery (blood samples and hemorrhage) and vein (blood samples and resuscitation, when used). After cannulation, the DSI telemetry unit was buried under several layers of muscle, and the animal was rotated onto its left side. The saline-filled catheters were tunneled subcutaneously to the dorsum over the shoulders and exteriorized. The incisions were closed with staples and infused with bupivacaine. Isoflurane was turned off, but mechanical ventilation was maintained, and the animal was placed in a sling with feet down. Limb electrocardiographic leads were attached, BIS electrodes (Bispectral Index; Aspect Medical Systems, Newton, MA) were placed across the forehead, stopcocks were placed in all of the catheters, and the sling was lowered until the animal's feet were on the floor. Midazolam infusion was started at 1.25 mg/kg/hr and adjusted throughout the study to maintain a BIS sedation level between 80 and 90. BIS is used to monitor electroencephalographic activity for depth of anesthesia or sedation, or both. A BIS level of 40 to 60 is considered adequate for surgical anesthesia[17] and 80 to 90 is appropriate for "conscious sedation" in humans. A warm water blanket and a warm air Bair Hugger were placed over the animal for temperature control.

As soon as the animal demonstrated adequate spontaneous breathing, the endotracheal tube was removed, and the animal was allowed to stabilize. After 30 minutes of stabilization, baseline hemodynamic data (systolic, diastolic, and mean blood pressure and heart rate [HR]) were collected, and baseline arterial and venous blood samples were taken for the following parameters: Po_2, So_2, Pco_2, HCO_3, base excess (BE), pH, Hct, Hb, glucose, lactate, differential white blood cells, and platelets, using standard clinical chemistry techniques. Total arterial and venous blood taken for analysis was 26 mL per sample.

Hemorrhage

Hemorrhage followed immediately after the baseline blood samples. Blood volume was estimated (EBV) to be 65 mL/kg (data from Sinclair Research Center, Columbia, MO). EBV was corrected for the blood samples taken before and during hemorrhage. The swine were exponentially hemorrhaged 60% of their corrected EBV for 60 minutes at 5 equal volumes over periods of 7.5 minutes, 11.5 minutes, 12 minutes, 13 minutes, and 16 minutes.[4] Arterial and venous blood samples were taken at the end of each hemorrhage period for

the same parameters as baseline. Hemorrhage volume and rate were computer controlled and adjusted for the arterial and venous blood samples taken during hemorrhage. The computer program was based on feedback control using LabVIEW (National Instruments, Austin, TX). The computer was programmed for the 5 different flow rates and hemorrhage volumes. A roller pump withdrew blood from the animal into a container on a balance. Blood volume was measured as weight on the balance using 1.038 as the specific gravity of swine blood. When the balance measured the programmed blood volume (weight), the pump was turned off through a feedback loop. Blood samples were taken, and the next flow rate and hemorrhage volume were initiated. The process was repeated to hemorrhage ~60% of EBV for 1 hour. Heparin was infused into the pump tubing (110 units/min) distal to the blood sampling site to prevent clotting in the roller pump withdrawal tubing. No heparin entered the animal.

After hemorrhage, arterial and venous blood samples were collected at 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes or until the animal died. Death was defined as respiratory arrest. Hemodynamic data were sampled continuously. No resuscitation fluids were provided. Any animal that survived >3 hours was killed with 10 mL of Fatal Plus (Dearborn, MI), intravenously.

Control

Throughout these studies (21 months), 16 nonresuscitated control animals were evaluated. There were 12 control animals studied during validation of the model and 4 contemporaneous control animals during evaluation of resuscitation products.

Hextend Resuscitation

After development of this model, it was important to determine whether the model could be resuscitated similar to previous models by using a standard of care resuscitation product. Two swine were used as contemporaneous controls and treated similar to the model development swine to assure that the model was still responding as originally determined. Fourteen swine were hemorrhaged similar to control swine and then resuscitated with Hextend (Hospira, Lake Forrest, IL) immediately after hemorrhage. Hextend (6% hydroxyethyl starch [colloid] physiologically balanced with electrolytes, lactate, and glucose), is a standard of care currently used as a prehospital resuscitation fluid by the US military.[18]

In addition to the computer controlled hemorrhage system mentioned earlier, additional feedback programming was developed for Hextend resuscitation to achieve hypotensive resuscitation.[18] The blood pressure signal from the DSI catheter in the carotid artery was patched to the computer. The resuscitation program controlled a second roller pump that infused Hextend into the femoral vein at 1 mL/kg/min to raise systolic blood pressure (SBP) to either 65 mm Hg ± 2 mm Hg (Hextend 65; n = 7) or 80 mm Hg ± 5 mm Hg (Hextend 80; n = 7). Additional Hextend was administered only if SBP fell below the specified limits. SBP was not controlled if it exceeded the specified limits. Infused Hextend was warmed to 37°C with a Level I System 1000 fluid warming device. The animals were observed and sampled for up to 6 hours at 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, 240 minutes, 300 minutes, and 360 minutes.

17β-Estradiol (E2) Resuscitation

Two swine were used as contemporaneous controls and treated similar to the model development swine to assure that the model was still responding as originally determined. Twelve additional swine were studied in two groups of six swine each, and hemorrhaged as described earliler. Immediately after hemorrhage, one group of six swine was given a 1 mg/kg bolus of E2 in normal saline, whereas the second group of six swine was given a 10 mg/kg bolus of E2 in normal saline. 17β-estradiol was obtained as water soluble cyclodextrin-encapsulated 17β-estradiol (Lot 066K1500; Sigma, St. Louis, MO). The compound (45 mg of estradiol/gm of compound) was dissolved in sterile saline and intravenously administered at room temperature for ~30 sec. The 1 mg/kg dose of E2 was dissolved in 40 mL of sterile saline and had an osmolality of 310 mOsm/L and a pH of 5.4, whereas the 10 mg/kg dose was dissolved in 50 mL of sterile saline with an osmolality of 485 mOsm/L and a pH of 6.75. No additional resuscitation was provided. The animals were observed for up to 3 hours and sampled similar to the control animals. Plasma E2 was measured from each blood sample from baseline through 180 minutes of recovery for the swine that received 1 mg/kg of E2 (ELISA Estradiol Kit 402110; Neogen Corporation, Lexington, KY).

Additional arterial and venous blood (total of 26 mL/sample/time point) was taken at the programmed times from the four groups after hemorrhage. Blood samples were replaced by ~10 mL to 15 mL of saline to flush the catheters.

Statistics

A within-groups repeated measures analysis of covariance was used to examine the difference between the control group (model development) and the two resuscitation groups (Hextend and E2), including change over time and the interaction of time and group. The hemorrhage and the resuscitation/recovery periods were considered separately in the analysis. If the repeated analysis of covariance showed a significant difference between groups over time, a post hoc analysis was performed to determine which time points were significantly different using an independent *t* test between the groups (or nonparametric equivalent, as necessary). A Kaplan-Meier estimate of survival was constructed to compare control versus Hextend and control versus E2 by the stratified log rank test. Values in the text are mean ± SEM, and $p \leq 0.05$ was considered statistically significant.

RESULTS

All 42 swine used in these studies were identically treated from baseline through the 60 minutes of hemorrhage, resulting in consistent data over that period with between group significant differences only in glucose and hematocrit at baseline (Table 1).

TABLE 1. Cardiovascular and Arterial Metabolic Data

	Baseline	End of Hemorrhage	90 Min Post Hemorrhage
Systolic blood pressure (mm Hg)			
Control	151 ± 5.0	38 ± 2.4	50 ± 19.4 (4)
Hextend 65	129 ± 4.6	45 ± 3.0	85 ± 4.4
Hextend 80	146 ± 11.8	25 ± 5.9	97 ± 6.6*
Estradiol	146 ± 7.2	52 ± 6.5 (11)	77 ± 9.7 (8)
Heart rate (b/min)			
Control	76 ± 4.7	179 ± 9.9	168 ± 29.2 (3)
Hextend 65	64 ± 3.7	166 ± 12.3	176 ± 13.8
Hextend 80	67 ± 6.3	180 ± 5.8	134 ± 14.4
Estradiol	70 ± 3.2	177 ± 9.3 (11)	205 ± 9.8 (8)
pO_2 (mm Hg)			
Control	85.4 ± 1.8	97.8 ± 2.7 (13)	93.4 ± 6.9 (5)
Hextend 65	82.1 ± 2.3	104.9 ± 3.0	97.6 ± 3.5
Hextend 80	86.4 ± 1.8	98.0 ± 4.3	94.4 ± 1.6
Estradiol	81.8 ± 1.9	95.2 ± 2.2 (11)	103.2 ± 3.4 (8)
pCO_2 (mm Hg)			
Control	46.7 ± 1.5	29.6 ± 1.8 (13)	30.8 ± 7.3 (5)
Hextend 65	48.8 ± 0.8	32.7 ± 1.6	35.0 ± 2.6
Hextend 80	47.8 ± 1.7	32.5 ± 1.9	42.6 ± 1.6
Estradiol	49.4 ± 1.4	34.9 ± 1.8 (11)	31.6 ± 2.6 (8)
pH			
Control	7.45 ± 0.01	7.46 ± 0.02 (13)	7.39 ± 0.03 (4)
Hextend 65	7.44 ± 0.00	7.43 ± 0.01	7.40 ± 0.02
Hextend 80	7.45 ± 0.01	7.44 ± 0.01	7.42 ± 0.02
Estradiol	7.44 ± 0.01	7.43 ± 0.01 (11)	7.36 ± 0.01 (8)
HCO_3 (mmol/L)			
Control	31.4 ± 0.6	20.7 ± 1.1 (13)	15.1 ± 3.4 (5)
Hextend 65	32.1 ± 0.5	21.1 ± 0.6	21.3 ± 2.1
Hextend 80	32.2 ± 0.9	21.4 ± 1.1	27.3 ± 1.9*
Estradiol	32.2 ± 0.5	22.4 ± 1.0 (11)	16.9 ± 1.6 (8)
Base excess (mmol/L)			
Control	6.5 ± 0.5	−2.0 ± 0.9 (13)	−9.8 ± 3.0 (5)
Hextend 65	7.2 ± 0.5	−2.2 ± 0.4	−2.9 ± 2.1
Hextend 80	7.1 ± 0.8	−1.9 ± 0.8	2.6 ± 1.9*
Estradiol	7.3 ± 0.4	−1.5 ± 1.0 (11)	−7.6 ± 1.8 (8)
Lactate (mmol/L)			
Control	1.0 ± 0.1	8.3 ± 0.9 (13)	14.9 ± 3.4 (4)
Hextend 65	1.0 ± 0.1	9.2 ± 0.5	11.2 ± 0.8 (6)
Hextend 80	0.9 ± 0.1	7.3 ± 0.6	7.5 ± 1.9
Estradiol	0.9 ± 0.1	6.8 ± 0.6 (11)	13.6 ± 1.5 (8)
Glucose (mmol/L)			
Control	5.1 ± 0.1	11.9 ± 1.0 (13)	7.2 ± 0.7 (5)
Hextend 65	4.9 ± 0.1	12.2 ± 1.7	8.4 ± 1.0
Hextend 80	4.3 ± 0.2*	10.7 ± 1.0	6.5 ± 0.6
Estradiol	4.3 ± 0.2*	9.3 ± 1.1 (11)	8.2 ± 1.1 (8)
Hematocrit (%)			
Control	41.6 ± 1.2	41.8 ± 1.4	39.6 ± 1.4 (5)
Hextend 65	33.6 ± 1.1 *	39.6 ± 1.3	34.6 ± 1.6
Hextend 80	38.1 ± 1.7	46.4 ± 2.9	26.6 ± 3.3*
Estradiol	37.0 ± 1.0 *	43.4 ± 1.8 (11)	41.9 ± 2.6 (8)

Data are mean ± SEM; n = 16 for Control, 7 for Hextend 80 and 65 and 12 for Estradiol, except where indicated by ( ). * = significantly different from control ($p < 0.05$). Data are not shown beyond 90 min post hemorrhage since there was only one Control animal that survived to the next data point (120 min).

Control—No Resuscitation

The response to hemorrhage of the four contemporaneous control swine (two from the Hextend groups and two from the E2 group) was similar to the previous 12 swine used for model development and thus were added to the control group (n = 16). The 60% hemorrhage of EBV from the 16 control swine averaged 38.8 ± 0.1 mL/kg (Table 2) and resulted in a decrease in SBP from 151 mm Hg ± 5.0 mm Hg to 38 mm Hg ± 2.4 mm Hg and an increase in HR from 76 bpm ± 4.7 bpm to 179 bpm ± 9.9 bpm (Table 1; Fig. 1). At the same time, there was a decrease in arterial P_{CO_2}, HCO_3, and BE and an increase in lactate and glucose (Table 1; Fig. 1). There was a slight compensatory recovery of SBP from end of hemorrhage (EOH) through 90 minutes and then a gradual decline resulting in death in most animals. HR remained increased, sometimes exceeding 200 bpm, until just before death when it decreased dramatically at approximately the same time as respiratory arrest. Fifteen of the 16 control swine (94%) died within 116 minutes after the EOH. Average survival time for the 16 animals was 64 minutes ± 11.5 minutes. One control swine survived for the entire 3 hours (6%).

Hextend Resuscitation

Six of the seven (86%) Hextend 65 swine and all seven (100%) of the Hextend 80 swine survived for 3 hours, whereas five of the seven (71%) Hextend 65 and six of the seven (86%) Hextend 80 survived for 6 hours after Hextend infusion. Average survival time for both Hextend 65 and Hextend 80 through 3 hours was significantly greater than control (Table 2; Fig. 2). The 60% hemorrhage from the 14 animals averaged 38.9 ± 0.1 mL/kg and was not different from control (Table 2). Changes in metabolic parameters at the EOH were similar to control (Table 1). The recovery of most parameters toward control values at 90 minutes post-hemorrhage was greater for Hextend 80 than Hextend 65 (Table 1; Fig. 1). The replacement volume of Hextend needed to increase and maintain a minimum SBP of 65 mm Hg ± 2 mm Hg and 80 mm Hg ± 5 mm Hg averaged 265 mL ± 34 mL (6.1 mL/kg ± 0.8 mL/kg) and 640 mL ± 109 mL (15 mL/kg ± 2.6 mL/kg), respectively (Table 2). Mean SBP at the EOH and the beginning of resuscitation was 38 mm Hg ± 2.4 mm Hg. SBP responded quickly to Hextend infusion. By 15 minutes, the Hextend 65 swine had reached the target SBP of 65 mm Hg (70 mm Hg ± 2.7 mm Hg), whereas SBP for the Hextend 80 swine was 79 mm Hg ± 3.9 mm Hg. By 30 minutes, all of the swine had reached their target pressure (Fig. 1). After resuscitation SBP for Hextend 65 and Hextend 80 was 83 mm Hg ± 1.9 mm Hg and 97 mm Hg ± 5.3 mm Hg at 1 hour, 87 mm Hg ± 4.9 mm Hg and 96 mm Hg ± 5.0 mm Hg at 3 hours, and 87 mm Hg ± 5.5 mm Hg and 107 mm Hg ± 11.3 mm Hg at 6 hours, respectively in surviving animals.

17β-Estradiol (E2) Resuscitation

The average survival times and survival rates of 117 minutes ± 23.6 minutes (1 of 6) and 133 minutes ± 21.2 minutes (2 of 6) for the two E2 groups of 1 mg/kg and 10 mg/kg, respectively, were not significantly different from

TABLE 2. Swine Weight, Age, Hemorrhage Volume, Hextend Volume and Survival Time

	Wt. (kg)	Age (Months)	Hem. Vol. (ml)	Hem. Vol. (ml/kg)	Hex. Vol. (ml)	Hex. Vol. (ml/kg)	Mean Survival (Min)	Survival at 180 Min
Control (n = 16)	40.4 ± 1.4	13.3 ± 0.5	1565 ± 53.7	38.8 ± 0.1			64 ± 11.5	1/16 (6%)
Hextend 65 (n = 7)	43.7 ± 0.8	11.7 ± 0.5	1702 ± 33.6	38.9 ± 0.07	265 ± 34	6.1 ± 0.8	171 ± 8.6*	6/7 (86%)*
Hextend 80 (n = 7)	43.0 ± 1.2	14.8 ± 0.9	1676 ± 44.7	38.9 ± 0.1	640 ± 109#	15 ± 2.6#	180 ± 0.0*	7/7 (100%)*
17β estradiol (n = 12)	47.1 ± 1.4*	13.1 ± 0.8	1821 ± 52.0*	38.7 ± 0.08			125 ± 15.3*	3/12 (25%)

Data are mean ± SEM. * = significantly different from control; # = significantly different from Hextend 65 ($p < 0.05$). Hem Vol = hemorrhage volume; Hex Vol = hextend volume. Percent shed blood volume replacement with Hextend = 16% for Hextend 65 and 38% for Hextend 80.

each other ($p = 0.59$; Fig. 3, A). Therefore, the two groups were combined as one group (n = 12) and statistically compared with the control group. The 60% hemorrhage from the 12 animals averaged 38.7 mL/kg ± 0.1 mL/kg and was not different from the control swine (Table 2). After hemorrhage, 3 of 12 swine (25%) survived for 3 hours after E2 administration. The other 9 animals died at an average of 107 minutes ± 16.2 minutes, resulting in an overall average survival time of 125 minutes ± 15.3 minutes for all E2 swine (Table 2). E2 average survival time was significantly greater than average control survival time (125 ± 15.3 versus 64 ± 11.4; $p = 0.01$) but percent survival at the target time of 180 minutes was not significantly different from control (25% versus 6%; $p = 0.16$; Table 2; Fig. 3, B). A power analysis indicated that for the 25% survival of the combined E2 groups at 3 hours to be significantly different from the 6% survival of the Control group at 3 hours would require a test of 65 animals in each group.

Mean plasma concentration of E2 at 15 minutes after E2 treatment of 1 mg/kg (n = 6) was 100 times greater than E2 concentration at baseline or EOH. Plasma E2 concentration decreased exponentially from the 15 minutes post-E2 sample but was still 10 times higher than baseline or EOH concentration at 180 minutes post-E2 treatment (n = 2).

Core temperature ranged from an average baseline temperature of 37.3°C to an average temperature of 37.2°C at 90 minutes posthemorrhage, across all animals.

DISCUSSION

A conscious, sedated, mature male miniature swine hemorrhage model has been successfully developed and resuscitated with a standard of care fluid (Hextend). In addition, the model was used to evaluate E2 as a potential small volume resuscitation product. The response to severe hemorrhage was consistent with previous studies in unanesthetized domestic swine[4] and was characteristic of a hemorrhage-induced metabolic acidosis, with a decrease in blood HCO_3, and BE and an increase in blood lactate (Fig. 1), suggesting a conversion to anaerobic metabolism in many tissues. However, mean arterial pH stayed >7.43 and increased, or showed little change during the later hemorrhage periods, even though buffering capacity was being depleted, indicative of respiratory compensation. There was an abrupt decrease in arterial pH after hemorrhage (Fig. 1). Although data from this study during hemorrhage were consistent with previous studies using unanesthetized immature domestic swine in a sling and hemorrhaged for 1 hour or anesthetized miniature swine hemorrhaged for 15 minutes, there were some differences. In this study (n = 42), average baseline mean arterial pressure (MAP) of 126 mm Hg ± 3.2 mm Hg was higher than previous conscious (99–107 mm Hg; n = 28), anesthetized (61–78 mm Hg; n = 48), or resting normal data[19] from immature domestic swine (102 ± 1.4; n = 44; Table 3). Also, average MAP of 35 mm Hg ± 2.2 mm Hg at EOH was lower than previous conscious swine EOH (Table 3) and closer to the anesthetized swine EOH. Moreover, baseline HR was lower than the other two animal models, whereas, EOH HR was similar for all three hemorrhage groups. Possible reasons for the increased baseline MAP in this study could be the anesthetic and surgical placement of catheters on the day of the study, in contrast to instrumentation 7 days to 10 days earlier in the previous studies, allowing time for adaptation.

Hextend is recommended by Tactical Combat Casualty Care guidelines for treating severe blood loss.[18] Although Hextend does not provide significant buffering or O_2 carrying capacity, it is a very effective plasma volume expander,[20] allowing increased tissue perfusion and oxygenation at reduced, but adequate, pressure and flow levels. Similar studies using anesthetized male and female Yucatan miniature swine that were hemorrhaged 40% to 55% of EBV for 15 minutes, and resuscitated with Hextend to a MAP of 60 mm Hg, required 29 mL/kg to 30 mL/kg of Hextend during prehospital (4 hours) resuscitation.[6,7] Survival ranged from 100% at 4 hours[6] to 75% to 88% at 72 hours.[6,7] The difference in fluid requirements (29–30 mL/kg versus 6–15 mL/kg in this study) may be related to the difference in hemorrhage time (15 minutes versus 1 hour). The 1 hour hemorrhage would allow more time for transcapillary flux of fluid into the vascular space in an attempt to maintain blood volume.

Endogenous E2 (the most important and prevalent estrogen) has been implicated in the protection of premenopausal women against hypertension and atherosclerosis, compared with males over the same age range.[21] In contrast, postmenopausal women have a dramatic increase in both hypertension and atherosclerosis at a time when endogenous estrogens are decreasing, suggesting a gender and age difference in vascular vulnerability.[21] Although E2 has been shown to protect female rats in proestrus and male rats given E2 (50 μg/kg) before, during, or after T-H,[15] the same level of protection was not observed in this swine study, even at the higher E2 dose of 10 mg/kg. Previous reviews have discussed both the genomic and nongenomic effects of E2.[22] The slower genomic response to E2 results in upregulation and down-

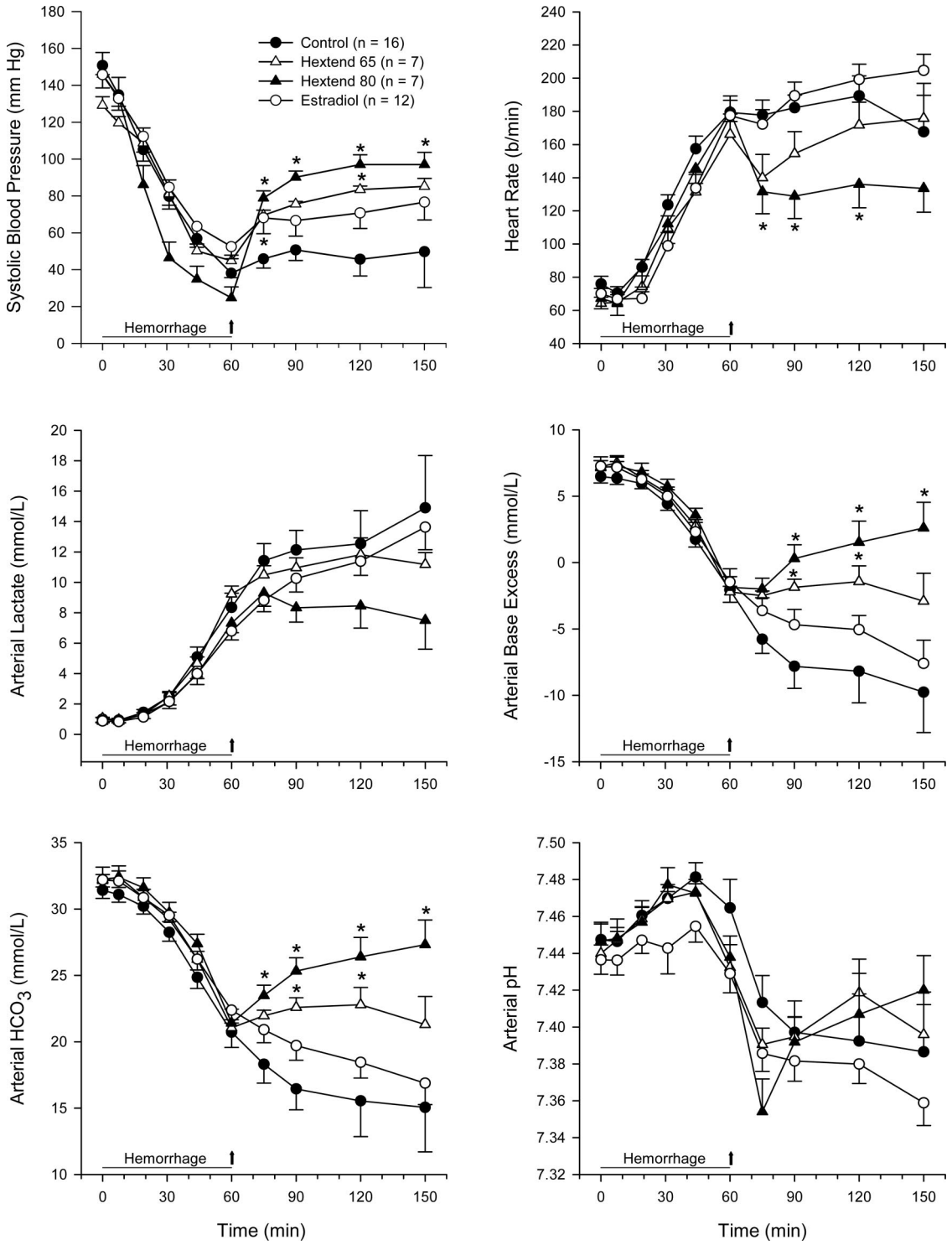

Figure 1. Blood pressure, heart rate and metabolic responses to a 60% hemorrhage over one hour followed by 90 min of recovery from the Control, Hextend 65, Hextend 80 and Estradiol groups. Data are mean ± SEM. * = significantly different from control ($p < 0.05$). Arrows indicate beginning of Hextend or Estradiol infusion.

regulation of numerous cellular functions producing cytokines and chemokines for control of immunity, inflammation, tissue damage, and apoptosis.[23–25] Vascular relaxation of rat aortic rings,[8] swine coronary rings,[13] and swine femoral vein rings[9] after E2 treatment are considered to be nongenomic E2 responses. Endothelium-independent[12] and dependent[9,10,13]

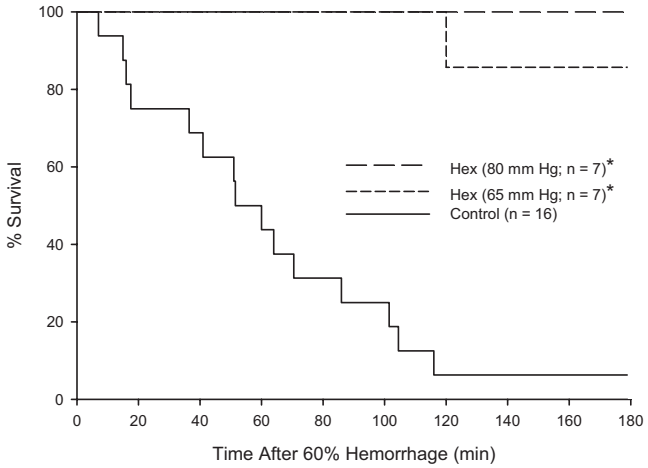

Figure 2. Kaplan-Meier survival plot of control data (n = 16) vs. Hextend 65 (n = 7) and Hextend 80 (n = 7) data plotted through 3 hours. *Indicates significantly different ($p < 0.05$) than control.

nongenomic vascular relaxation mechanisms have been proposed requiring activation of cyclic AMP,[12] release of nitric oxide,[9,10,13] and reduction of vascular smooth muscle intracellular Ca^{++}.[11] Therefore, the acute, rapid, nongenomic cardiovascular effects of E2 could explain the significant improvement in the E2 swine average survival time compared with control ($p = 0.01$) observed in this study (Fig. 3, B); however, the survival benefit was not sustained, and E2 survival at 180 minutes was not different from control.

Hemorrhage protection after E2 is most likely an integrated combination of both genomic and nongenomic responses—the complete protection scenario is still unknown. Also unknown is the genomic contribution toward survival during the 180-minute recovery period. The failure of significant E2 protection at 180 minutes in this male swine hemorrhage study compared with rodents could be related to differences in animal strain, body size, metabolism, insufficient time for genomic contribution, or reduced ERs and ER agonists. It has been demonstrated in humans that there are less ERs in the male than the female,[21] and after puberty, there is a decrease in plasma nitric oxide (a strong vasodila-

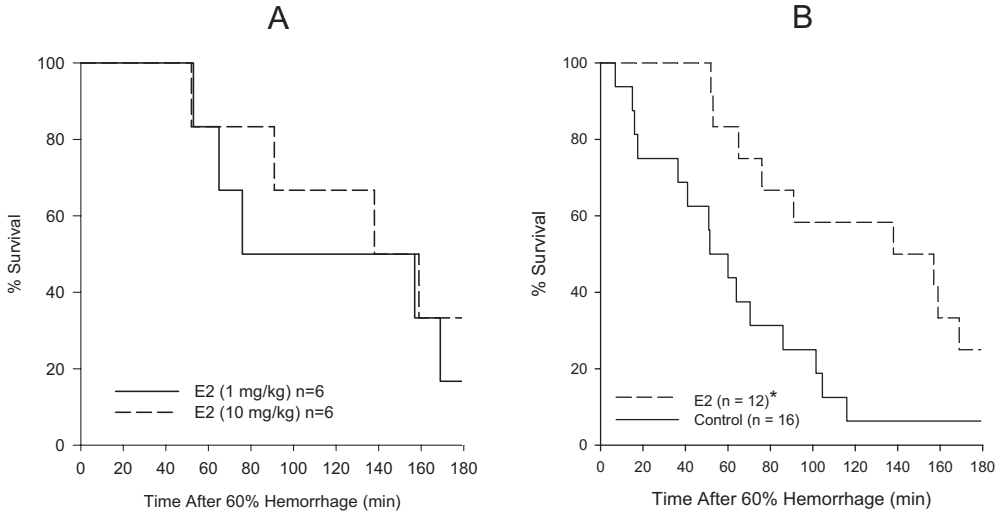

Figure 3. Kaplan-Meier survival plot. (A) E2 doses of 1 mg/kg and 10 mg/kg. There was no significant survival difference between the two doses ($p = 0.59$). Therefore, data from the two groups were combined and compared to the control group. (B) Control data vs. E2 (n = 12). *Indicates significantly different ($p < 0.05$) than control. Although average E2 data was significantly different than control, it was not significantly different than control at the target time of 180 minutes ($p = 0.16$).

TABLE 3. Hemodynamic Comparisons

	Current Study—Sedated (Sinclair Miniature Swine; n = 42)	Previous Conscious Hemorrhage (Domestic Swine; n = 28)	Previous Anesthetized Hemorrhage (Yucatan Miniature Swine; n = 48)	Resting Normals (Domestic Swine)
MAP (mm Hg)				
Baseline	126 ± 3.2	99–107	61–78	102 ± 1.4 (n = 44)
EOH	35 ± 2.2	52–62	20–26	
HR (bpm)				
Baseline	71 ± 0.24	110–116	122–145	105 ± 2.7 (n = 15)
EOH	177 ± 5.0	170–204	161–170	

Data are mean ± SEM for current study and resting normals[19]; previous conscious[6] and anesthetized[7–9] data are ranges of means. The previous conscious swine were bled ~58% of EBV for 1 hr, whereas the anesthetized swine were bled 40–55% of EBV for 15 min.

tor) and E2 in the male swine,[26] indicating that vascular reactivity in the adult male swine may not be as responsive as in the female.

Many of the rodent hemorrhage studies were resuscitated with fluids in addition to the E2; distinctly different from this swine hemorrhage study where no additional fluids were provided. The Hextend-treated swine in this study were given either 6.1 mL/kg ± 0.8 mL/kg or 15.0 mL/kg ± 2.6 mL/kg of Hextend for resuscitation, whereas the E2 swine were given 1 mL/kg to 1.5 mL/kg of saline as the carrier for the E2. It is unknown whether 6 mL/kg to 15 mL/kg of additional saline given to the E2 swine would be beneficial because saline is a very transient volume expander. Also, in this study, body temperature was controlled, in contrast to earlier rat studies, in which the rats were allowed to become hypothermic, providing a possible survival advantage.[14]

Plasma from the swine receiving 1 mg/kg of E2 immediately after hemorrhage yielded a mean E2 concentration well above the baseline and EOH plasma concentration, suggesting that the plasma concentration of E2 in this swine study should have been adequate to elicit a significant nongenomic response. The plasma concentration decreased exponentially in the following samples but was still increased at 180 minutes. Moreover, the 10 mg/kg dose of E2, although not measured, should have resulted in a plasma E2 level >1 mg/kg dose. The doses of 1 mg/kg and 10 mg/kg of E2 did provide a significant increase in the average survival time (Fig. 3, B) but not at the target time of 180 minutes. The significant improvement in E2 average survival time is possibly a nongenomic E2 response, which was not sustained throughout the 180 minutes. Higher concentrations of E2 may have resulted in improved survival.

Study Limitations

Controlled hemorrhage has been criticized as being unrealistic. Severe hemorrhage, generally, is the result of severe trauma resulting in uncontrolled hemorrhage and tissue injury. However, controlled hemorrhage without trauma allows for more precise evaluation and comparison of different resuscitation products.

The 180-minute survival target time limitation is driven by the anticipated evacuation times for removal of severely injured personnel from the Afghanistan forward conflict areas or from remote domestic locations.

The small volume bolus infusion of 1.0 mL/kg to 1.5 mL/kg of E2 into an animal that has lost 60% of blood volume is considered a high survival risk. The distribution of the E2 to critical organs without additional fluid resuscitation is severely limited by reduced organ and peripheral blood flow.

Summary

A conscious, sedated, severe hemorrhage model has been developed using the sexually mature Sinclair male miniature swine. The animals were hemorrhaged 60% of their EBV exponentially for 1 hour with an average survival time of 64 minutes ± 11.5 minutes and a survival rate of 6% at 180 minutes posthemorrhage, without resuscitation. The model was successfully resuscitated with Hextend to a SBP of 65 mm Hg ± 2 mm Hg or 80 mm Hg ± 5 mm Hg (hypotensive resuscitation) resulting in a respective 86% and 100% survival rate at 180 minutes. Resuscitation with E2 was only marginally successful. Average survival time for E2 resuscitation was significantly greater than control (125 minutes ± 15.3 minutes versus 64 minutes ± 11.5 minutes; $p < 0.03$), but the E2 survival rate of 25% at the target time of 180 minutes was not different from control ($p = 0.16$).

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